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Microorganisms associated with the hindgut of *Oniscus asellus* (Crustacea, Isopoda)

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With 3 figures

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1. Introduction

In woodlice, nutrients are thought to be absorbed from the hindgut (Sutton 1980) and in particular from the papillate region of the hindgut (Hassall & Jennings 1975). In comparison with the ingested food the hindgut contains a greater density of microorganisms (Coughtrey *et al.* 1980; Hanlon 1981; Anderson & Ineson 1983) which are considered to be important in the breakdown of food material (Hassall & Jennings 1975). Bacteria adherent to the hindgut cuticle have also been observed (Bignell 1984) and these may, therefore, be important in digestion or decomposition. In this study we describe the distribution and properties of microorganisms adherent to the hindgut cuticle of *Oniscus asellus* L.

2. Materials and methods

2.1. Preparation of *O. asellus* hindguts

Specimens of *Oniscus asellus* L. were collected as required from the Macaulay Institute grounds. They were anaesthetised by chloroform vapour and transferred to sterile distilled water where the hindgut and rectum were removed by gripping the head and pleon with sterile tweezers and pulling firmly apart. The hindgut was cut away from the rectum with a sterile scalpel.

2.2. Enumeration of microorganisms in the hindgut

The hindguts of 5 *O. asellus* were homogenised separately in 5 ml of sterile distilled water using a tissue grinder. Triplicate plates were prepared from each hindgut homogenate using a spiral plater (Don Whitley Scientific Ltd., Shipley, England), on both Tryptone Soya Agar (Oxoid) (TSA) and Starch-Casein Agar (Kuster & Williams 1964) containing nystating (50 $\mu g \cdot ml^{-1}$) and cycloheximide (50 $\mu g\ ml^{-1}$) (SCA). Bacterial colonies were counted on TSA after 2 days, and actinomycete colonies counted on SCA after 14 days incubation at 25 °C. Hindgut dry masses were determined by filtering a 3 ml aliquot of hindgut homogenate through preweighed membrane filters of 0.3 μm diameter pore size, and drying the filters to constant mass at 80 °C.

2.3. Scanning electron microscope (SEM) observations of hindgut cuticle

Hindguts of 5 *O. asellus* were fixed for 2 h in a solution of glutaraldehyde, 3% v/v in 0.1 M phosphate buffer, *p*H 6.8 at 20 °C, rinsed in buffer for 1 h and then dehydrated in a water-ethanol-freon series (Kinden & Brown 1975). The hindguts were cut longitudinally while in freon and the gut contents removed. The dissected hindguts were critical point dried, attached to aluminium stubs with colloidal carbon, coated with gold and examined in a Cambridge S4 stereoscan.

2.4. Growth of adherent bacteria in culture

The gut contents were removed from 10 hindguts, by forcing water through the hindgut with a sterile syringe, and the hindguts homogenised in 10 ml of sterile distilled water. Aliquots (1 ml) of homogenate were added to flasks containing 9 ml of either sterile distilled water, 1% m/v nutrient broth (Oxoid) or a gut extract prepared by homogenising 100 *O. asellus* hindguts in 50 ml distilled water and filtering through a membrane filter with 0.3 μm diameter pores. Sub-samples (1 ml) were taken from 3 replicate flasks of each medium at intervals up to 6 h at 25 °C, and plated on TSA as in section 2.2.

2.5. Metabolic activity and growth of bacteria on hindgut cuticle

Five hindguts were cut open, cleaned as in section 2.4 and stained to demonstrate dehydrogenase activity with 0.2% m/v 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) for 10 min at 20 °C. The reaction was then stopped with 4% formaldehyde and hindguts mounted in water and examined microscopically.

Unstained hindguts, prepared as above, were mounted aseptically in water and colonies of adherent bacteria, observed with phase-contrast microscopy, were photographed at regular time intervals. The increase in the size of individual colonies with time was determined as the increase in the area of their photographic image measured with an Optomax system 111 image analyser (Micro Measurements Ltd., Saffron Walden, England).

The most frequently isolated bacteria from section 2.4., subsequently identified as *Enterobacter agglomerans* and an isolate of *Pseudomonas fluorescens* obtained from soil were cultured separately in nutrient broth (Oxoid) containing 0.02% m/v INT for 18 h at 25 °C. The bacteria were concentrated and washed by aseptically centrifuging the culture at 582 g for 5 min. The supernatant was discarded and the pellet resuspended in sterile distilled water five times. Hindguts cut open and cleaned as in section 2.4 were mounted in the final bacterial suspension for microscopical examination. Bacterial cells labelled with the INT-formazan were located on the cuticle and their increase in size determined as above.

2.6. Identification of bacteria

Twenty morphologically distinct bacterial isolates from the culture experiment (section 2.4.) and from unstained hindguts observed in section 2.5. were cultured on nutrient agar (Oxoid). The isolates were identified using diagnostic tests for gram -ve bacteria (HARRIGAN & MCCANCE 1966).

3. Results

3.1. Enumeration of microorganisms in the hindgut

The five specimens had a mean bacterial density of 2.657×10^5 mg^{-1} dry mass $\pm$ 2.367 (S.D.), and a mean actinomycete density of $3.762 + 10^4$ mg^{-1} dry mass $\pm$ 3.023 (S.D.).

3.2. SEM observations of hindgut cuticle

Rod shaped bacteria were observed on the hindgut cuticles examined, in colonies of various sizes. One of the largest colonies observed is shown in Fig. 1 (a & b). The cuticular surface was sparsely colonised by bacteria, which were not associated with any particular region of the hindut and had no regular pattern of attachment. No filamentous organisms were observed.

3.3. Growth of bacteria in culture

The bacteria in both nutrient broth and gut extract entered a logarithmic growth phase after 2 h incubation. Their instantaneous growth rate (STAINER *et al.*, 1971) in nutrient broth was 6.06 h^{-1} $\pm$ 0.148 (S.D.), which was significantly different ($P < 0.01$) than 4.56 h^{-1} $\pm$ 0.108 (S.D.) in gut extract. There was no growth in the sterile distilled water control.

3.4. Metabolic activity and growth

Colonies of rod shaped bacteria were observed on the hindguts examined. The bacteria occurred infrequently but were more common on the papillate region of the hindgut (Fig. 2a & b) than on the anterior hindgut where there is a greater density of cuticular spines. The bacteria observed were dehydrogenase active, as demonstrated by the presence of INT formazan within the bacterial cells. The increase in size of the 14 colonies measured was used to calculate the instantaneous growth rate of 0.213 h^{-1} $\pm$ 0.106 (S.D.).

E. agglomerans labelled with INT-formazan grew when inoculated on to the hindgut cuticle (Fig. 3a & b) at the same rate as naturally adherent bacteria. Thirteen colonies measured

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Fig. 1. Scanning electron micrographs of a colony of rod-shaped bacteria on the hindgut cuticle. The bacteria arrowed in 1a (bar = 10 μm) are shown in greater detail in 1b (bar = 1 μm).

Fig. 2. A colony of naturally adherent bacteria growing on the hindgut cuticle next to cuticular spines (CS). Mircograph 2b was taken 175 min after micrograph 2a at 25 °C. Bar = 3 μm.

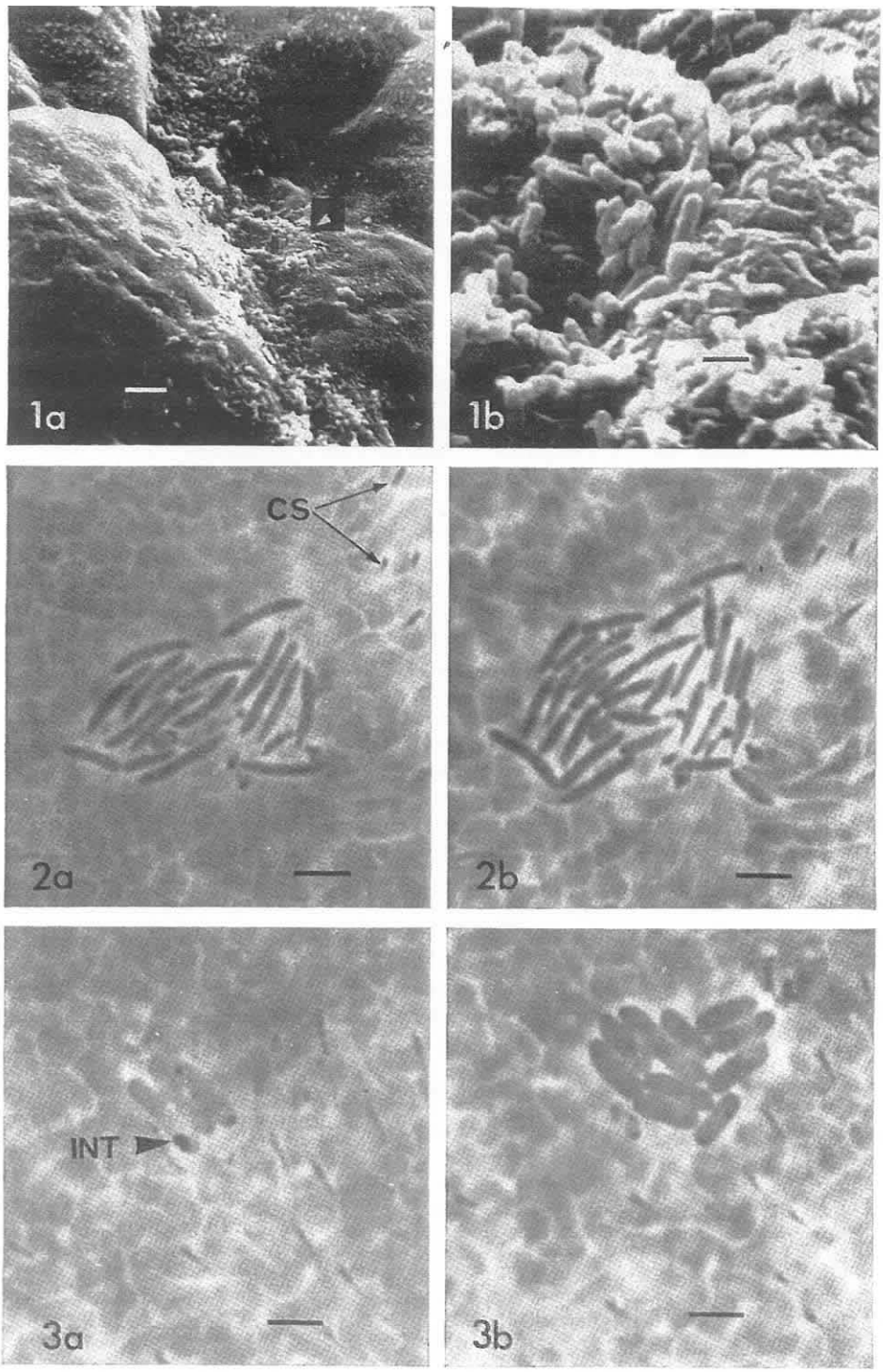

Fig. 3. Cells of *E. agglomerans* labelled with INT growing on the hindgut cuticle. Micrograph 3b was taken 188 min after micrograph 3a at 25 °C. Bar = 3 μm.

had an instantaneous growth rate of 0.222 $h^{-1} \pm 0.147$ (S.D.). The 18 colonies of labelled *P. fluorescens* cells measured had an instantaneous growth rate of 0.045 $h^{-1} \pm 0.035$ (S.D.) which is significantly less ($P < 0.001$) than that of either *E. agglomerans* or the natural adherent bacteria.

3.5. Identification of bacteria

The bacteria isolated from cleaned *O. asellus* hindguts were identified as *Pseudomonas* (Pseudomonadaceae), *Plesiomonas* (Vibrionaceae), and Enterobacteriaceae. The numerically dominant organism, isolated from culture experiments (section 2.4) was identified as an anaerogenic strain of *Enterobacter agglomerans*, most closely resembling the subgroup 1X of BRENNER *et al.* (1984).

4. Discussion

The observed density of microorganisms in the hindgut is of the same order of magnitude as reported by COUGHTREY *et al.* (1980), who counted 1.3×10^5 bacteria and 2.1×10^4 actinomycetes gut^{-1}. Given a gut dry mass of 1.2 mg, as in this study, these figures would be equivalent to 1.08×10^5 bacteria and 1.75×10^4 actinomycetes mg^{-1}. ANDERSON & INESON (1983) recorded a higher density of bacteria (2.8×10^6 mg^{-1}) in the hindgut of *O. asellus*. No filamentous microorganisms were observed on the hindgut, although they are present on the gut cuticles of millipedes (BIGNELL 1984), termites (BIGNELL *et al.* 1979), cockroaches (BRACKE *et al.* 1979) and tipulid larvae (KLUG & KOTARSKI 1980) for example. Thus, any actinomycete activity in *O. asellus* would be restricted to the gut contents but the actinomycetes isolated on agar plates may have been present as spores rather than hyphae (MAYFIELD *et al.* 1972).

Pseudomonas sp. are the dominant bacteria in the hindgut of the woodlouse *Tracheoniscus rathkei* (REYES & TIEDJE 1976) and were isolated from *O. asellus* in this study. The common soil bacterium *P. fluorescens* grew significantly less well on excised hindgut tissue than the naturally adherent bacteria. This indicates that there may be some degree of adaptation to the gut environment by the naturally occurring adherent bacteria.

Light and scanning electron microscope observations both demonstrate that the hindgut is colonised by bacteria. These bacteria are metabolically active, as shown by their ability to reduce a tetrazolium salt using an enzyme system (dehydrogenase in this instance) necessary for electron transport (MACDONALD 1980). Their sparse and irregular distribution suggests that if any bacteria in the hindgut are important in digestion, as concluded by HASALL & JENNINGS (1975), then they will be bacteria ingested with the food rather than those adherent to the hindgut cuticle. The adherent bacteria may simply be exploiting the nutrients released during digestion and absorbed across the hindgut cuticle.

5. Acknowledgements

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6. References

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Synopsis: *Original scientific paper*

Griffiths, B. S., & S. Wood, 1985. Microorganisms associated with the hindgut of *Oniscus asellus* (Crustacea, Isopoda). Pedobiologia **28**, 377—381.

The hindgut of *Oniscus asellus* L. contained an average of 2.66×10^5 bacteria mg^{-1} and 3.76×10^4 actinomycetes mg^{-1}. Scanning electron and light microscopy demonstrated the presence of rod shaped bacteria adherent to the cuticle of washed hindguts, which were shown to be metabolically active. The most frequently occurring bacteria isolated from culture was *Enterobacter agglomerans*, with other Enterobacteriaceae, *Plesiomonas sp.* and *Pseudomonas sp.* also present. The common soil bacterium *Pseudomonas fluorescens* grew significantly less well on excised gut tissue than the naturally adherent bacteria, suggesting adaptation by the resident microflora. The bacteria adherent to the hindgut cuticle were not present at high enough densities to influence the breakdown of organic matter.

Key words: Woodlice, *Oniscus asellus*, gutflora.